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Pain Vulnerability: A Neurobiological Perspective

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Abstract

There are many known risk factors for chronic pain conditions. Yet the biological underpinnings that link these factors to abnormal processing of painful signals are only just beginning to be explored. This review will discuss the potential mechanisms that have been proposed to underlie vulnerability and resilience towards developing chronic pain. Particular focus will be given to genetic and epigenetic processes, priming effects on a cellular level and alterations in brain networks concerned with reward, motivation/learning and descending modulatory control. While research in this area is still in its infancy, a better understanding of how pain vulnerability emerges has the potential to help identify individuals at risk and may open up new therapeutic avenues.

Introduction

Considerable advances have been made in understanding the neurobiology of chronic pain over the last two decades. The molecular mechanisms leading to amplification of pain-related signals in chronic pain states have been dissected. An unexpected contribution of non-neuronal cells in the CNS has been discovered, and functional as well as structural neuroimaging studies have revealed a brain organization and plasticity unanticipated by previous animal studies.

While the field is thus edging closer towards the "How", one major unresolved question is "Why", or more particularly "Why me?" A ubiquitous patient's lament, to which neuroscience may be able to provide some answers. An emerging body of evidence highlights neurobiological processes that could render some individuals more vulnerable or more resilient to developing chronic pain and this will be the focus of this review.

There are many examples in the clinical literature demonstrating that only a proportion of patients with a particular disease or injury go on to develop chronic pain (Table 1): diabetic neuropathy is a relatively common condition, but only a minority of patients report pain as one of their symptoms; a subset of individuals undergoing operations develop chronic pain (between 5% and 40% depending on the type of surgery); about a third of lower back pain sufferers go on to develop a persistent syndrome lasting for 12 months or more; and finally, quite surprisingly, no strong relationship can be found between pain and joint damage in osteoarthritis, despite extensive study [2-5].

What is different between chronic pain sufferers and those that escape this fate? Epidemiological studies of some of the patient groups described above have identified several risk factors that may predispose towards the condition. Some of these are intrinsic to the individual, such as gender, age and genetic make-up. Women are more likely to develop certain chronic pain conditions, as are older people, although in some instance age may function as a protective factor. The influence of genetics is supported by twin and population-based studies, which clearly indicate that painful conditions and acute pain sensitivity per se are heritable (see Crow et al. for a recent review [7]). Other risk factors relate to an individual's personality and psychosocial environment. Not surprisingly, previous pain history predicts future pain development. But additionally, adverse life events, such as stress and unemployment, as well as personality traits, a tendency to catastrophize, and depressive illness negatively affect long-term pain outcome. While the presence of these links is not in doubt, cause and effect often remain unclear.

It is not our intention to discuss these risk factors in any depth – the interested reader is referred to [2-4] - but rather to consider the mechanisms by which they may impact the emergence or maintenance of chronic pain (Figure 1). Their elucidation might not only help identify individuals at risk, but also deepen our understanding of persistent pain conditions and potentially open up new avenues for the development of preventative and targeted treatment regimes.

Genetic risk

Human genetic studies have had a dramatic impact on many branches of medical science, including pain. There have been two distinct approaches which will be discussed in turn: linkage analysis in families suffering from rare Mendelian disorders in which single gene mutations cause profound loss- or gain-of-function, and association studies in large cohorts, where genetic variants are correlated with differences in a particular trait, such as height, or in the current context, pain sensitivity.

A number of families have been identified that show monogenic patterns of inheritance for sometimes dramatic pain phenotypes, such as complete analgesia or extreme pain. Congenital analgesia is rare, with an estimated prevalence of about one in a million, and the precise symptoms and underlying genetic mutations vary between families [101]. Yet, their study has not only revealed the mechanism by which risk is conferred in these particular individuals, but has also deepened our understanding of chronic pain in the general population. For instance, congenital insensitivity to pain with anhidrosis (HSAN-IV, CIPA) is due to recessive loss-of-function mutations in the TRKA receptor gene (see [13] for review). This result helped consolidate pre-clinical findings which have implicated TRKA and its ligand NGF in nociceptor sensitization [16] and has eventually led to both targets being pursued by the drug development industry, with promising results: tanezumab, an NGF antibody has reached phase III of clinical trials for the treatment of hip and knee osteoarthritis, and may also be effective in other chronic pain conditions, such as back pain and interstitial cystitis (see http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/ArthritisAdvisoryCommittee/UCM295205.pdf). Similarly, a linkage study of a Chinese family in 2004 identified a completely novel target in primary erythermalgia, the sodium channel subunit $Na_v1.7$ (SCN9A). Mutations in SCN9A can also cause indifference to pain and paroxysmal extreme pain [17]. Animal studies have since confirmed the presence of $Na_v1.7$ in 85% of nociceptors and its importance for processing both mechanical and inflammatory painful stimuli [17]. Several sodium channel blockers are now in phase IIa clinical trials to test their efficacy against pain of diverse etiologies. Finally and most recently, another sodium channel subunit has emerged as a potential target, with a gain-of-function mutation having been reported in Nav1.9 (SCN11A) as another cause of pain insensitivity [13].

In contrast to rare Mendelian conditions, the study of pain genetics in the wider community presents a more complex picture. What everyone can agree on is that a sizable degree of risk is indeed accounted for by genetics: most heritability estimates from twin studies range from 13-60% depending on the pain phenotype and cohort examined [7], and even in the general population they can reach 30% for severe chronic pain [18]. As to identifying the genes responsible, the pain field has mostly conducted case-control candidate gene association studies that have revealed a wide variety of risk alleles. Loci for which a positive association has been reported are involved in neurotransmitter systems (COMT, OPRM1, GCH1, 5HTR2A, ADRB2), ion channel (KCNS1, CACNA2D3) and immune function (IL1, TNF) [101]. For most of these, the mechanistic steps by which any single nucleotide polymorphism (SNP) or haplotype identified might confer risk towards chronic pain in later life are not very clear, although more functional, pre-clinical studies are beginning to emerge, e.g. [12, 19]. More worryingly, as summarized in more detail by Mogil recently [101], results are often not replicable, not least because of issues with poor phenotyping, population stratification and sample size.

Recently, genome-wide association studies (GWAS) have been employed, providing unbiased

screening of common variants. However, many of the GWAS studies published, while examining painful disorders, such as osteoarthritis [20], lumbar disc degeneration [21] or endometriosis [22], barely mention pain in their articles, let alone measure it directly. There are notable exceptions: several large scale GWAS and a meta-analysis in migraine research [23]; a study of molar extraction, which we note only examined acute post-surgical pain and may have been somewhat underpowered with only 100 participants [24]; a study of opioid sensitivity which revealed a SNP close to the *CREB1* gene [25]; and a GWAS meta-analysis of chronic wide-spread pain syndrome. The latter study merged and re-analysed previously collected genotyping data to identify novel variants in two genes (CCT5 and FAM173B), the expression of which was found altered in a mouse model of pain [26].

What could be improved to help elucidate the genetic risk factors for chronic pain? A fundamental question that remains and the answer to which will greatly influence study design is whether many genes will be linked to nociception and pain per se (such as SCN9A). There are two, not mutually exclusive alternatives: risk haplotypes might differ according to the various underlying painful disorders, and hence future research effort should focus on GWAS such as the above-mentioned on osteoarthritis. Conversely, genes might be more strongly linked to the various nociceptive modalities such as thermal or mechanical hypersensitivity, independent of the original source of the pain. Evidence from animal models indicates that modality can be more important than the underlying condition, but data from humans remains contradictory [101]. In either case, rigorous, standardized phenotyping, e.g. using quantitative sensory testing, will be required to advance the field, not only for accurate pain modality assessments, but equally to help generate homogeneous cohorts with little stratification. Likewise, family-based designs provide greater protection from the latter. They can also facilitate the exploration of rare variants, by helping to distinguish them from sequencing errors, as evinced by a recent exome sequencing study [27], which found that heat pain sensitivity in twin pairs was associated with a regulatory network around angiotensin II.

Taking into account interactions, both on a phenotype and genotype level could make another improvement. Studies often neglect to collect phenotype data on confounding factors that could modulate pain, such as anxiety and depression and therefore are in danger of wrongly assigning risk to biological pathways unrelated to pain. Moreover, epistatic effects, i.e. interactions between genes, as well as interactions between genes and the environment are commonly ignored, although studies in mice [28] and more recently humans [29] clearly indicate that they can play an important role.

Finally, research into the genetics of pain should not stop at identifying the putative casual allele. While still rare, there now are examples of studies that move into the functional realm with evidence-based examination of potential biological consequences. A genome wide linkage analysis in mice identified a haplotype in the P2X7 receptor gene associated with strain-specific variations in hypersensitivity to mechanical stimuli. The authors then carried out pre-clinical work and showed that the risk haplotype was associated with a structural change in the ion channel pore of P2X7, which had consequences for nociceptive processing. Lastly, they identified a corresponding haplotype in humans that was associated with two distinct pain syndromes (post-surgical pain and osteoarthritis)[30]. Other instances in which studies bridge the mechanistic gap between gene and behaviour can be found in the brain imaging literature [31], where individual genotypes have been related to changes in activity in relevant cortical areas. Thus, functional polymorphisms that are weakly related to chronic pain syndromes might be strongly related to the integrity of the underlying neural systems as revealed by brain imaging. However, it is not easy to ascribe causality at this stage. Studies have focused on polymorphisms that influence catecholamine and serotonergic neurotransmission (i.e. COMT and 5-HTT and see [31] and references therein) – reflecting a link to reward and the descending pain modulatory system (DPMS), which will be discussed in more detail below. COMT appears to be more involved in paradigms that model chronic and tonic, rather than acute pain and has been reported to have widespread effects on affective and cognitive tasks mediated by the prefrontal cortex (PFC). However, SNPs in the gene could not be convincingly related to overall pain risk in some genetic studies [31], and it may therefore only have relevance

during the expression rather than the development of chronic pain due to the PFC related effects. In contrast, imaging studies on serotonin receptor and transporter systems may be more promising with a view to identifying potential vulnerability. Neuroimaging studies that associate 5-HTT with the experience of pain in healthy individuals and patients are emerging and again are discussed more extensively in a recent review [31]. Additionally, genetic variation in 5-HTT has been linked to altered brain frontal-limbic network reactivity to relevant environmental stimuli and a predisposition to several neuropsychiatric disorders. Within the anxiety literature, it is interesting how many parallels with pain exist in terms of genetic polymorphisms and environmental stressors influencing PFC-amygdala networks (see Bishop and references therein [32]) that might confer vulnerability to both conditions.

Pain vulnerability: Epigenetics

The previous section examined how differences between individuals' DNA sequences can predispose towards pain, but what about differences in how this sequence is used? Epigenetics studies phenomena such as DNA methylation and histone modifications, which do not affect the sequence itself, but can affect gene function – a kind of biological annotation mechanism. Epigenetic signatures determine lineage-specificity during development and can be stably maintained throughout the life of an organism and in some cases even across generations, e.g. in the case of imprinting of parental alleles [33].

In chronic pain, some associations to epigenetic markers have recently been identified. Thus, altered methylation was observed at the PARK2 locus in patients with lumbar disc degeneration [21]. Moreover, back pain was also found to be linked to methylation changes at the SPARC gene in both humans and mice [34].

It is currently not clear how much of this putative variation is present from birth and how much is acquired later. Until a decade ago, dogma on DNA methylation maintained that, in healthy tissues, the modification remained mostly unaltered postnatally. However, research since has identified mechanisms for active DNA demethylation [35], and evidence is accumulating to suggest that both DNA methylation and histone modifications can change rapidly in an adult organism, even in a postmitotic environment [36]. Epigenetic modifications may therefore provide a way in which environmental influences can leave a long-term imprint on gene expression. The idea, first proposed by so-called behavioral epigeneticists, has encountered a healthy level of skepticism [37], but is gaining traction from research in many other fields, including normal brain function, aging, and a variety of disorders such as neurodegeneration and chronic pain [38, 39]. In the current context, the hypothesis is that injury or disease might result in a type of molecular memory that could affect a person's risk of developing chronic pain at a later stage.

Evidence in support of this hypothesis is starting to emerge slowly. Histone modifications seem to play a role in both inflammatory and neuropathic pain conditions, as evinced by the analgesic effect of histone deacetylase inhibitors – drugs that interfere with the removal of histone acetyl groups [39, 40]. There are also first indications that changes in histone modification may correlate with changes of expression of relevant genes [7], although the direction of causality remains unclear, as does the biological significance of histone marks at individual genetic loci [41]. In the case of DNA methylation, one early drug study, using a DNA methyltransferase inhibitor, reported alleviation of hypersensitivity after chronic constriction injury [42]. However, these results are inconclusive, due to their use of a compound unable to act on postmitotic cells [43]. Correlational work has been carried out, linking global changes in DNA methylation in the PFC and amygdala to peripheral nerve injury [44], and examining local alterations in DNA methylation at several genetic loci [7, 45]. Again, cause and consequence are unknown. Finally, the most substantial body of work has been conducted around the methyl-CpG-binding protein 2 (MECP2), an enzyme crucial to neuronal development, which binds to methylated cytosine-phosphate-guanine motifs (CpGs). MEPC2 is downregulated after nerve injury in the dorsal root ganglia, its targets upregulated in the spinal cord

after peripheral inflammation [46], and mutations in its sequence can lead to abnormal pain sensations in patients [47]. Work from Skene et al. has suggested that MECP2 binds neuronal DNA very widely and might function as a global regulator of chromatin remodeling, recruiting co-repressors to the right place at the right time, thereby reducing transcriptional noise [48]. One could therefore hypothesize that even subtle differences in MECP2 function might have noticeable consequences and could, at least in theory, be at the root of inter-individual differences in phenotype.

To summarize, the literature on pain and epigenetics is still in its infancy. Only a small number of papers have been published so far and, not surprisingly for such a novel field, some of them still suffer from basic technical issues. These include lack of negative controls for chromatin immunoprecipitation and use of compounds better suited to dividing cell systems. What does seem clear is that persistent pain states are associated with epigenetic modulation of histones or DNA, and that drugs targeting epigenetic processes can modify pain processing. What is unknown is whether long-term vulnerability or resilience for pain arises from these processes. For future work, the cell-type specificity of epigenetic marks will be a major task to address, especially when it comes to DNA methylation studies in humans. In the case of histone modifications, timescales, especially in post-mitotic cells, and precise function still need to be elucidated. It may be worthwhile to bear in mind evolutionary conservation [49] and the high degree of redundancy when considering their biological significance. Long-term risk for chronic pain may be more likely to be conferred by differences in DNA methylation, arguably one of the most stable epigenetic marks. Focus should be on studying cell-specific models in which causality can be established. New compounds for the study of epigenetics are continually emerging and may greatly aid this work.

Priming mechanisms

The previous section has argued that epigenetic mechanisms might confer risk for chronic pain by functioning as a type of molecular memory – a record of prior injury or disease that may adversely affect future responses to similar insults. But is there any evidence that such a priming mechanism does indeed exist in chronic pain? There are several lines of research that indicate that early life stressors or even previous injury in adulthood can make an animal more vulnerable to develop persistent pain. We will discuss postnatal experiences and adult priming in turn.

Pain exposure in early life can lead to heightened pain sensitivity once the animal has fully developed. This has been shown for diverse stimuli, such as neonatal chronic footshock, inflammation and incision (see [50] and references therein). Moreover, not just pain, but also early-life stress seems sufficient to induce hypersensitivity in later life. Thus, maternal separation can lead to increased visceral hypersensitivity in adult rats [51] and mice [52]. Many potential mechanisms have been proposed for these phenomena, including alterations to the opioid system, increased axonal sprouting or NGF-induced neuronal plasticity, involvement of the hypothalamic-pituitary-adrenal axis and of spinal microglia [50, 53, 54]. Most recently, imaging data have confirmed these pre-clinical findings. Studies on preterm infants examined at various time-points after hospital discharge confirm that alterations in brain processing occur and that this impacts cognitive outcomes [55] and brain reactivity specifically to painful events [56].

In adulthood, priming has been induced using low dose inflammatory stimuli that ordinarily only result in short lasting hypersensitivity. When a rat is administered two consecutive low doses, the second one will cause longer-lasting (days rather than hours) and more pronounced hypersensitivity (see Figure 1B). Priming can be observed with diverse inflammatory mediators, such as prostaglandin E2, serotonin and NGF, and with stress caused by unpredictable sound [57]. The phenomenon has also been reported with other paradigms employing repeated nerve injury, stress prior to nerve injury or formalin- and injury-induced enhancement of pain following intrathecal lipopolysaccharide (LPS) injection (see [58] and references therein). Mechanistic explorations of priming fall into two main categories, focusing on peripheral afferents and spinal microglia, respectively. Experiments from the Levine laboratory indicate that the priming stimulus activates an

additional PKCε-mediated second messenger cascade in isolectin B4-positive peripheral afferents. This in turn recruits the cytoplasmic polyadenylation element binding protein (CPEB), a regulator of protein translation, which is then hypothesized to render nociceptors more responsive to pro-inflammatory cytokines [59]. A second strand of work studied alterations to microglial responses in the spinal cord, following the induction of priming [58]. For example, minocycline, an inhibitor of microglial activation, was found to reduce priming induced by LPS injections in the rat.

'Vulnerable' Brain Networks

Having discussed possible molecular and cellular risk factors, it is important to now ask whether brain networks play a role. The concept that differences in brain function relate to both individual variances in behavior and perhaps a 'vulnerability' towards or 'resilience' against causing a diseased or chronic pain state is not new outside the pain field [32, 60, 61]. However, data are relatively sparse, as firm agreement on what a "normal brain" looks like and how networks relate to mechanisms is lacking. Moreover, the traditional approach in brain imaging is to group average results, and hence smooth out any variances. Despite these caveats, discussed in more detail in Text Box 1, there are several studies reporting inter-individual differences in brain activity, structure, wiring and chemistry. They specifically relate to endogenous modulatory capacity [62], psychological traits [63], pain thresholds in healthy subjects [64, 65] and patients [66], clinical descriptors [67] or opioid analgesic outcomes [68].

What remains unclear is whether these brain correlates of trait and behavioral variance in healthy subjects translate into an increased likelihood for developing chronic pain. Understanding whether changes in brain networks are consequential to having chronic pain or causal in producing it is very difficult and relies on detailed, longitudinal knowledge of biological and environmental subject variables. We lack a definitive answer, but data discussed here suggest there might be several candidate 'causal' networks, summarized in Figure 1C. We will focus our discussions on the reward-motivation-learning network and the DPMS.

The reward-motivation-learning network

A recent study from Baliki and colleagues [69] comes closest to performing the 'pre- to post injury' longitudinal imaging study that is ideally needed. They performed a longitudinal brain imaging study of subacute back pain patients over the course of one year using a battery of brain imaging measures from the acute pain phase onwards. Pain persisted in twelve patients at the one-year time stamp, while twelve patients improved. In the persistent pain group, gray matter density was decreased, as shown to occur in other chronic pain conditions. But of particular relevance are the results from the first 'baseline' imaging session during the acute pain phase. Here, greater functional connectivity or 'coupling' of the nucleus accumbens (NAc) with the PFC predicted pain persistence by more than 80%. This implies that corticostriatal circuitry might be causally involved in the transition from acute to chronic pain. Interestingly, this increased coupling remained constant throughout the transition to chronic pain despite gray matter density decreasing within the NAc. In an additional analysis the authors discovered brain white matter connectivity differences within the PFC at an early time point, which again was more pronounced in the group that went on to develop chronic pain. These changes may reflect structural 'vulnerabilities', as measured by diffusion tensor imaging (DTI) and fractional anisotropy (FA) calculations. Importantly, as with the functional connectivity measures, these white matter FA differences accurately predicted pain persistence over the next year, and this was validated in a second cohort of subacute back pain patients [70]. While it is unknown whether these differences in function and structure were present pre-injury and hence represent an a priori risk for pain, this study nevertheless highlights how the brain's reward-motivational learning circuitry is potentially relevant in predicting the transition from acute to chronic pain. In an earlier study, Baliki and colleagues had already reported results that hinted at a possible 'bias' in the reward network prior to chronic pain development [71]. That study showed

differential NAc responses to acute noxious thermal stimuli in controls and chronic back pain patients implying an altered valence to acute pain exists between patients and controls.

Indeed, studies in the past have noted the relevance of reward circuitry in pain [72], and other related networks, such as those relevant to dopaminergic signaling, have also been involved. Thus, patients with fibromyalgia have disrupted dopaminergic reactivity [73]. Further, placebo analgesia in healthy controls can be predicted by dopamine-related traits, with its magnitude correlated to gray matter density in the insula, ventral striatum, and PFC [74]. A link between the ability to experience analgesia and the brain reward network is also supported by findings from our laboratory. Baseline responses to a painful stimulus were found in reward networks, involving for example the ventral tegmental area and the NAc. This baseline activity was predictive of both subsequent opioid induced behavioral analgesia and its neural expression via the DPMS [68].

Despite these fascinating results, the precise role of the reward-motivation-learning system in pain remains unclear and may depend on context. We showed that the hedonic value of pain can be 'flipped', fundamentally altering its emotional value from threat to reward. This change was mediated by activity in reward regions working in concert with the DPMS [75], providing further evidence for the importance of these networks in pain appraisal – a key feature of ongoing, chronic pain states. Dispositional optimism and pessimism, key trait factors relevant in pain, powerfully influence unexpected reward/analgesia outcomes with diametrically opposite NAc activity distinguishing the pessimists from optimists (discussed in [75]. Combined with data already discussed, it seems likely that transition to and continuation of chronic pain is dependent on the state of motivational/learning and reward mesolimbic-prefrontal circuitry of the brain.

The descending pain modulatory system (DPSM)

The DPMS is a powerful network that regulates nociceptive processing within the dorsal horn of the spinal cord and thereby controls what signals enter the brain. As such it plays a significant role influencing what pain you ultimately experience [8, 76]. The brainstem's component of the DPMS involves, among other nuclei, the periaqueductal gray (PAG) and the rostral ventromedial medulla (RVM). There is bidirectional central control of nociception that can either alleviate pain in situations where antinociception is necessary for survival (driven by "off" cells), as in sporting competition or battle, or can facilitate nociceptive processing (driven by "on" cells) and thereby contribute to the maintenance of heightened pain states. This was confirmed recently in several brainstem-imaging studies of chronic pain and central sensitization, a key dorsal horn event that amplifies incoming nociceptive inputs [77]. The anterior cingulate cortex, amygdalae and hypothalamus are also part of the DPMS, and these connections to the brainstem are the means by which cognitive and emotional variables interact with nociceptive processing to influence the resultant pain experienced, as shown in a wealth of brain and spinal cord imaging studies [78-80]. Neurochemically, the DPMS releases noradrenaline (NA) and 5-hydroxytryptamine (5-HT) on to spinal circuits. NA acts through its inhibitory alpha-2 adrenoceptor to inhibit, whereas 5-HT has bidirectional effects – inhibiting via 5-HT1 receptors and facilitating when 5-HT2 or 5-HT3 receptors are activated at spinal levels [8]. The polymorphisms in 5-HTT discussed earlier that influence pain outcomes are likely mediated via this system. Further, disturbances in sleep or mood, as well as early life stressors that are known to relate to neuroticism and anxiety could have profound developmental influences on this key system via alterations in the coupling of the amygdala-PFC network to the brainstem nuclei. Such an unfavorable 'imbalance' in inhibitory and facilitatory (i.e. off/on cell) drive could therefore predispose individuals towards developing persistent pain. Supporting data for this hypothesis comes from both recent animal and human studies.

One experiment measured patients' responses to painful stimuli in a laboratory setting and showed that results from certain tests could be used to predict acute pain after thoracotomy surgery [81]. Most predictive was pain temporal summation, i.e. an individual's level of pain in response to a series of heat stimuli. This measure is thought to be mediated by central processes like the DPMS and may represent 'neuroplasticity potential'. An alternative manipulation thought to tap into latent DPMS function via 'diffuse noxious inhibitory control' (DNIC) mechanisms is 'conditioned pain

modulation' [82], which Yarnitsky and colleagues showed could be used to predict lower risk of chronic post-thoracotomy pain [83]. In a more recent study, they found that poor DNIC efficiency predicts duloxetine efficacy in painful diabetic neuropathy [84]. Duloxetine targets the serotonergic and noradrenergic brainstem systems central to the DPMS and even 'corrected' the aberrant DNIC efficiency. While no imaging counterpart to these studies has been performed to verify the neural network 'at risk', it is highly likely defects in the DPMS inhibitory/facilitatory arms will be identified and relate to chronic pain transition.

In fact, animal studies suggest that this might be the case. Porreca's group has collected evidence to suggest that changes within the DPMS are crucial to the persistent nature of pain in models of nerve injury. They found that post injury decreases in descending inhibitory and increases in descending facilitatory activity on dorsal horn processing strongly influenced whether chronic pain behavior was maintained (and opposite for improved pain symptoms) [85, 86]. Knowing whether such an imbalance pre-exists ahead of injury is key, and evidence from neonatal rat studies might shed light on this issue. Hathway and colleagues showed, in a series of experiments that up to rat postnatal day 21, the RVM exclusively facilitates spinal pain transmission. However, after this age (postnatal day 28 to adult), its influence shifts to biphasic facilitation and inhibition [87]. These data hint at the possibility that should there be damage at a critical period of development (e.g. through stress or injury), it could permanently influence the 'set point' of the DPMS and possibly pain network maturation. The authors also demonstrated that there is another critical period for DPMS functioning during preadolescence [88], where a developmental transition from RVM descending facilitation to inhibition of pain occurs, which is determined by activity in central opioid networks. Their subsequent work showing how early life nerve injury produces a mechanical hypersensitivity only later in life is intriguing in the light of these findings [89].

In sum, these results lead us to the following hypothesis (yet to be tested): early life injury may create an imbalance in the DPMS, leading to inappropriate inhibition or facilitation of ascending pain signals. This in turn may create vulnerability and, as such, impact the maintenance of chronic pain states.

Hormones and the adolescent brain – a vulnerable time?

As noted from the animal studies above, there is a critical period of development during preadolescence. Although imaging studies examining how hormones generally influence brain activity are scarce, those published to date hint at the possibility that adolescent brains might be rendered vulnerable at this stage of hormonal upheaval [90]. Results support a link between the stress system and the DPMS, with one study showing that testosterone influences DPMS activity during altered estradiol states [91]. Other studies showed that repeated episodes of pain associated with menstruation throughout adolescence and early adulthood can be linked to central sensitization, alterations in brain function, structure and duration [92, 93]. A related line of research explores how sex differences might confer differential vulnerability, and several studies show significant sex-related structural differences in pain related regions [94]. This whole area is fertile for further exploration and we believe will play an increasingly important role in 'brain pain-vulnerability' related questions.

Can we outline a causal trajectory from aberrant brain activity?

As mentioned previously, a major caveat of the literature to date is its failure to identify causality. In addition to the studies already described that focus on the reward and DPMS networks, other studies are also trying to address this issue. They are still restricted to the injured state, but are taking a different approach and attempting to characterize whether 'non-pain' related features are present that correlate with differential brain activity or structure compared to controls. For instance, researchers have examined the contribution of a potential pre-existing vulnerability due to neuroticism, a stable personality trait characterized by a propensity for negative affect. Neuroticism was found to be positively correlated with increased thickness in the orbitofrontal cortex, an area linked to TMD-associated pain [95]. Similarly, a correlation between white matter connectivity strength

and neuroticism has been found in irritable bowel syndrome (IBS). And finally, IBS patients with a tendency to catastrophize their pain showed reduced dorsolateral PFC cortex thickness and increased hypothalamic grey matter (see [96] and references therein). These studies suggest that an individual's personality might be associated with differential brain structure and connectivity in areas relevant to chronic pain and that this might constitute vulnerability prior to the development of the condition that contributes to emergence and/or maintenance of the chronic pain state.

An additional phenomenon that has been examined in this context is attentional focus in the face of competing stimuli (e.g. having to perform a challenging cognitive task while experiencing pain). Thus, a recent study from Erpelding and Davis [97] classified subjects as 'pain focused' or 'attention focused'. Whether their data reflect vulnerability towards developing chronic pain remains to be determined, but promising parallels can be drawn to the anxiety literature. There, we know that frontal brain regions are involved in attentional regulation of emotionally and non-emotionally salient stimuli, including the dorsal and ventrolateral PFC and the rostral and dorsal anterior cingulate cortices. Some of these areas were differentially regulated in Erpelding and Davis' experiment, suggesting potential vulnerability in emotion regulation.

Conclusions

The literature leaves little doubt that certain groups of people are more vulnerable to develop chronic pain conditions. Evidence and viable hypotheses can be found as to why genetics and adverse priming events, such as a prior injury or stressful environmental influences, may confer increased risk. The latter may involve changes to neuronal architecture and molecular processes via epigenetic modulation that ultimately lead to changes in cortical wiring, brain chemistry, function and structure. Whether measureable alterations in brain function precede and/ or follow the onset of chronic pain, they might lead to a vicious cycle where 'vulnerability' leads to 'non-resilience' to additional factors arising from the chronic pain state. Possible support for this comes from several studies showing accelerated gray matter loss in chronic pain patients as if undergoing premature aging [98].

The characterization of brain imaging signatures in pain-free individuals prior to any injury will be crucial if we are to identify the relevant 'vulnerable' networks. Two current large-scale projects afford this opportunity: the UK's Imaging Biobank (http://www.ukbiobank.ac.uk) and The Human Connectome Project (http://humanconnectome.org). They are designed to use advances in neuroimaging, while simultaneously collecting in depth phenotypic and genotypic data from cohorts of healthy subjects, and in some instances following subjects longitudinally. Their outcome will provide a rich platform for future investigations linking structural and functional vulnerability and resilience to disease. They should also afford the chance to develop early-life interventions for improved well-being or better 'brain resilience', as perhaps illustrated in a recent study highlighting the benefits of yoga on brain circuits linked to increased pain tolerance [99].

The desire to identify and understand the biological underpinning of risk factors is often motivated by the hope for more targeted or preventative treatments. Indeed in the case of chronic pain it may be possible to use a combination of brain related measures, quantitative sensory testing and genotyping to aid stratification and improve treatment selection and targeting of interventions. We are not there yet, but recent imaging data points towards this being feasible [68]. Finally though, it is important to remember that stochastic and non-linear, chaotic processes have a major role to play in a person's life. Smoking causes cancer, but is neither a necessary nor a sufficient factor [100]. The goal of predicting who will develop chronic pain and who will be spared is a worthy one, but whether this is achievable at an individual level remains to be seen.

Text Box 1: **Brain Networks for Pain and its Modulation**

It is tempting to hypothesize that all networks subserving the emergence of pain perception and its modulation might contribute to the vulnerability towards or resilience against chronic pain development. However, most data come from pain studies in healthy controls and use repeated and short lasting stimuli more akin to acute pain. The networks identified might not always relate to chronic pain or be incomplete, as evinced in recent studies [1]. Advances in our ability to image within an individual ongoing or tonic pain states more relevant in chronic pain have occurred and look promising despite the technical and analytical challenges [6]. Such studies will provide additional opportunities to identify relevant 'vulnerable' networks.

Alongside these identified caveats, it should also be noted that brain imaging is not simply a surrogate 'objective' measure of pain ratings, but rather a very powerful tool for aiding the explanation behind why a subject reports their pain in a specific way. It can shed light on the many processes and factors that ultimately give rise to the individual experience of pain; namely an identifiable and measurable nociceptive drive, the immediate context, a person's emotional and cognitive state – and perhaps in future, an individual's brain vulnerability.

Interpretation is key, and most studies have been careful to use paradigms that dissect the activity from a complex network of responsive brain regions to associate regional activity with the various components that make up the multidimensional pain experience. Thus, non-specific responses in regions with roles in e.g. attention, expectation, anxiety and other emotions can be better understood neuroanatomically and in light of their contribution to pain experiences [8-10]. Therefore, the fact that many of the brain regions found active are not pain specific is not a new concept, and recent studies again highlight this point, but argue for the non-specificity to be considered instead as a brain network encoding the saliency of pain due to its predominance amongst many stimuli [11].

The advent of non-invasive tools has nevertheless been invaluable in increasing our understanding of the brain regions that subserve the private, multidimensional experience of pain. The current framework for the neural basis of pain perception includes a large bilateral network that is potentially available for activation - summarized in Figure 1C. Its different components can show varying levels of activation and can be recruited for activation (or not) in a dynamic fashion contingent on nociceptive drive, context, cognition, and emotion. If any of these factors change, the same nociceptive input can produce a different cerebral signature within the same subject, even stimulus by stimulus. Therefore, the behavioural reaction to such pain experiences is very efficient, being based on a rapid and adaptive brain response that is tailored to specific situations [8].

Additionally, this large network can be broken down into multiple interacting pain matrices of increasing neural hierarchy, as recently put forward by Garcia-Larrea and Peyron [14]. Multivariate pattern analysis has been used in an attempt to simplify this complex set of interacting networks to a core set of brain regions or a generalizable "pain signature". Such approaches identify the following areas as key to experiencing pain: the thalamus, the posterior and anterior insulae, the secondary somatosensory cortex, the anterior cingulate cortex and the periaqueductal gray matter [15] – still a complex pattern with the specificity question unresolved. Whether network differences in the acute or chronic pain networks are causal towards or consequential of chronic pain is not yet known. However, data from recent studies, and discussed in this review, suggest that several networks, including the reward-motivation-learning and descending pain modulatory system, might be aberrant pre-injury and confer a vulnerability towards developing chronic, persistent pain.

	Patient Statistics	Condition/Surgery	Incidence
Diabetes	15,692	Total incidence of neuropathy	48%
		Painful neuropathy	34%
Postsurgical pain	159,000	Amputation	30-50%
	479,000	Breast surgery	20-30%
	unknown	Thoracotomy	30-40%
	609,000	Inguinal hernia repair	10%
	598,000	Bypass surgery	30-50%
	220,000	Caesarean section	10%
Lower back pain	448	Pain 5 years after first presentation – prospective study	36.9%
	180	Pain 12 months after initial consultation – prospective study	34%
Neck Pain	5277	Incidence of chronic neck pain in cohort of patients reporting at least one episode of acute neck pain – prospective study	18%

Table 1: Examples of studies examining the emergence or incidence of chronic pain

Only a minority of acute pain sufferers, disease affected and surgical patients will develop chronic pain [2-4].

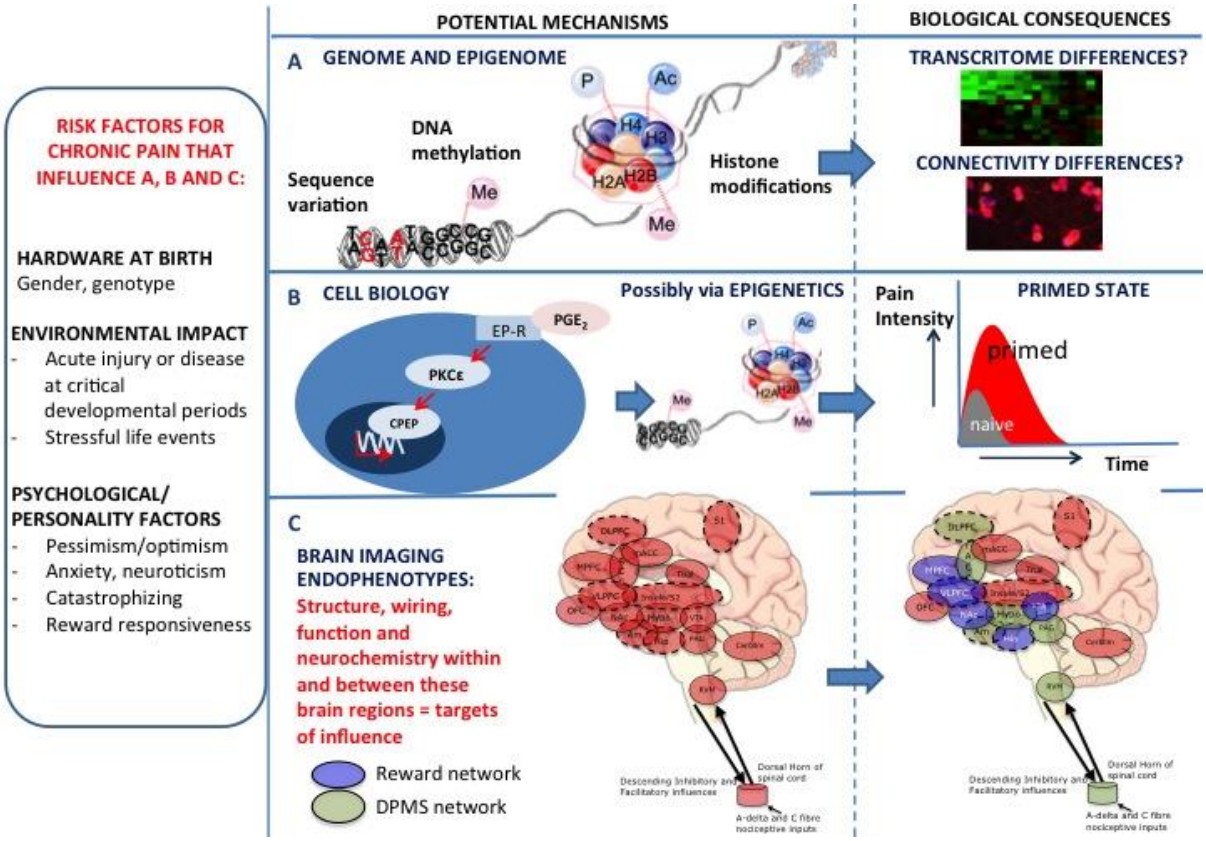

Figure 1: Risk factors for chronic pain, their potential mechanisms and biological underpinnings. Various risk factors have been identified for chronic pain, such as genetic, environmental and personality factors (left panel). Evidence for potential mechanisms underpinning these risk factors (middle panel) as well as their biological consequences (right panel) is beginning to emerge.

(A) Polymorphisms in the DNA sequence and epigenetic mechanisms such as DNA methylation and histone modifications determine some risk from birth that can lead to transcriptome and connectivity differences. Shown here is a schematic of DNA with two single nucleotide polymorphisms (SNPs in red) and modified by methylation (Me) at a CpG island. The DNA is wrapped around a histone octamer consisting of two H2A-H2B histone dimers and one H3-H4 histone tetramer, the lysine residues of which can be biochemically modified. Represented here are phosphorylation (P), acetylation (Ac) and methylation (Me).

(B) Environmental influences have been shown to cause changes at the cell biology level that may be consolidated through epigenetics to cause priming of the nociceptive system. Shown here is a putative intracellular cascade initiated by repeated prostaglandin E2 (PGE2) injection into a rat paw. PGE_2 binds the ephrin receptor (EP-R), and in a primed state causes activation of protein kinase C epsilon ($PCK\varepsilon$), which then may have transcriptional effects via the cytoplasmic polyadenylation element-binding protein (CPEB). In a primed state, pain intensity and duration in response to a nociceptive stimulus are increased compared to the naive situation.

(C) Many brain regions and networks contribute to normal and chronic pain experiences (red regions). Various brain networks may be involved in conferring vulnerability to painful conditions, in particular the reward-motivation network (purple regions) and the descending pain modulatory system (green regions). Evidence has been found for differences in structure, wiring, function and neurochemistry. (rACC/mACC (rostral/medial anterior cingulate

cortex); vlPFC (ventrolateral prefrontal cortex); dlPFC (dorsolateral prefrontal cortex); mPFC (medial prefrontal cortex); OFC (orbitofrontal cortex); insula/S2 (insular and secondary somatosensory cortex); S1 (primary somatosensory cortex); NAc (Nucleus accumbens); Am (amygdala); Hip (hippocampus); Hypo (Hypothalamus); Thal (thalamus); PAG (periaqueductal gray); RVM (rostral ventromedial medulla); VTA (ventral tegmentum) and Cerbllm (cerebellum)).

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